Effects of Electrical Stimulation on Osteoblasts on Anodized Nanotubular Titanium for Orthopedic Applications

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Statement of Purpose: The limited ability of titanium implants to remain bonded to juxtaposed bone and the extensive callus (fibrous tissue) formation surrounding such implants are two reasons leading to orthopedic implant failure. To improve the cytocompatibility properties of titanium, nanotechnology has been used to create nanotopographical features (through anodization) on titanium. Additionally, electrical stimulation has been investigated in orthopedics to heal bone non-unions and fractures. In this study, electrical stimulation was combined with anodized nanotubular titanium to further improve implant function**.** To investigate this, osteoblast (OB; bone-forming cell) and fibroblast (FB; fibrous tissue forming cells) functions were determined on anodized nanotubular titanium under various voltages for up to 21 days of in vitro culture. Moreover, to assess the extent of fibrous tissue formation, up to 5 day co-culture experiments were completed using OB and FB at various seeding densities.

Methods: 99.2% pure titanium foils were anodized using 20V DC current for 6 minutes with a 1.5% HF electrolyte solution and a platinum cathode and were characterized using scanning electron microscopy. OB (ATCC 11372, p#8-15) were cultured in DMEM supplemented with 10% FBS and 1% P/S and FB (ATCC 1213, p#6-12) were cultured in EMEM supplemented with 10% FBS under standard culture conditions. The total seeding density for co-culture experiments was 1500 cells/cm^2 (750/750). For the electrical stimulation experiments, bipolar pulses (0.4ms pulse duration and 20 Hz frequency) were used each day for 1 hour. The voltages used for these experiments were 1, 5, 10 and 15V, corresponding to 0.3, 1.4, 2.8 and 4.2 A/m^2. All cell experiments were completed for three times analyzed using the standard analysis of variance techniques.

Results: The results show that through anodization of titanium, a nanotubular oxide film was successfully created (Figure 1).

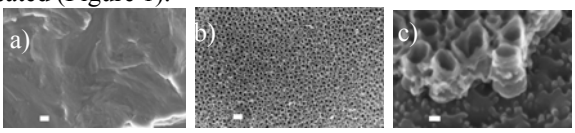

Figure 1: SEM images showing the surface features of a) conventional, b) anodized nanotubular and c) cross-section of the anodized nanotubular titanium. Scale bars are a) and b) 200nm, c) 30 nm.

OB proliferation experiments upon electrical stimulation (Figure 2) showed that under 15V of electrical stimulation, anodized nanotubular titanium had the highest cell density. When long term OB functions (Figure 3) were assessed using 15V of electrical stimulation, it was found that coupling the effects of an oxide nanotubular titanium morphology and electrical stimulation gave the most improved OB cell functions with respect to non-stimulated conventional titanium. In addition, the anodized nanotubular titanium showed lower FB density after 5 days of culture (data not shown). Finally, the co-culture experiments indicated that the anodized nanotubular samples stimulated with 15V had higher OB and similar FB density with respect to the non-stimulated conventional titanium samples (Figure 4). Similar results were also observed when the seeding densities were varied to 1OB-2FB and 2OB-1FB (data not shown).

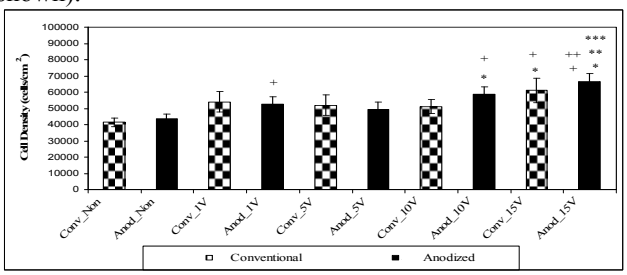

Figure 2: OB proliferation upon electrical stimulation at day 5. Values are mean ± SEM, n=3, *p<0.05 compared to Anod_Non, **p<0.05 compared to Anod_1V, ***p<0.05 compared to Anod_5V, +p<0.05 compared to Conv_Non, ++p<0.05 compared to Conv_10V.

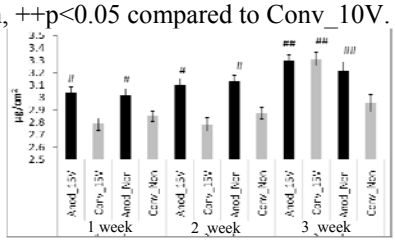

Figure 3: Calcium deposition after 1, 2 and 3 weeks of culture. Values are mean ± SEM, n=3, #p<0.05 compared to its conventional titanium counterpart at week 1 and 2. ##p<0.05 compared to 3 week Conv_Non.

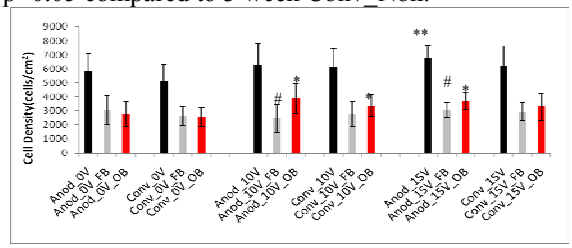

Figure 4: OB, FB and total cell densities upon electrical stimulation after 5 days. Values are mean ±SEM, n=3, *p<0.05 with respect to conv_0V_OB, #p<0.01 compared to osteoblast counterpart at the same voltage, **p<0.05 compared to conv_0V.

Conclusions: The combined effect of electrical stimulation and the creation of a nanotubular oxide film enhanced OB functions and at the same time decreased FB proliferation (possibly leading to less callus formation) important for improving titanium for orthopedic applications.

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